In our study, we investigated the biophysical properties of hemichannels Cx26 and Cx43 which were isolated biochemically and reconstituted into synthetic lipid membranes. Both hemichannels are present in different tissues and involved in different pathologies. The results on a study of the Cx26 are presented. Reconstitutions of functional Cx26 and mutant hemichannels were performed. Secondly, Cx43 was purified and reconstituted into bilayers. The hemichannel Cx43 properties were compared to previous studies and showed similarities of conductance on single channel recordings of Cx43 in cells. Our focus was then to form artificial gap junctions, first between two unrelated cells and then between cells and bilayers containing functional hemichannels. This was done using Cx26 or Cx43. The bilayer-cell configuration allows to measure electrophysiological properties of the cells indirectly via gap junctions. Single channel recordings of gap junctions were recorded using a bilayer containing Cx43 and Cardiomyocytes expressing Cx43. Macroscopic currents were as well recorded between bilayers and cell lines expressing Cx26 or Cx43.

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Asparagine175 of Cx32 is a Critical Residue for Docking and Forming Functional Heterotypic Gap Junction Channels with Cx26

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Gap junctions result from the docking of two hemichannels. Depend on the connexin(s) in the hemichannels, homotypic and heterotypic gap junction channels can be formed. Previous chimera studies with domain exchange between different connexins indicated that the extracellular loop2 (E2) is important molecular domain to heterotypic compatibility. Based on the high resolution structure of Cx26 gap junction channel and homology models of heterotypic channels, we analyzed docking selectivity for several hemichannel pairs, and found that the hydrogen bonds between E2 domains are conserved in several heterotypically compatible hemichannels, e.g. between Cx26 and Cx32. According to our model analysis, Cx32 mutation, Cx32N175Y, destroys three hydrogen bonds in the E2-E2 interactions at the heterotypic docking interface. Our model predicts that the Cx32N175Y hemichannel is unlikely to dock with Cx26 hemichannel properly due to steric hindrance at the docking interface. Experimentally, we tagged GFP and RFP to the carboxyl terminals of Cx32 and Cx26 to generate Cx32GFP (Cx32N175YGFP) and Cx26RFP, respectively. Our data showed that tagged Cx26 and Cx32 were able to traffic to cell interfaces forming gap junction plaque-like structures in transfected HeLa/N2A cells. However, Cx32N175YGFP exhibited mostly intracellular distribution and rarely seen in cell-cell interfaces. Double patch clamp analysis demonstrated that this Cx32 mutant did not form functional homotypic channels. When wild-type or mutant Cx32GFP expressing cells were cocultured with Cx26RFP expressing cells, Cx32GFP and Cx26RFP were frequently colocalized at the cell-cell interfaces and formed functional Cx32/Cx26 heterotypic channels. No colocalization was found at the cell-cell interfaces between Cx32N175Y and Cx26 expressing cells, also no functional Cx32N175Y/Cx26 heterotypic channels were identified. Our modeling and experimental data indicate that N175 of Cx32 is a critical residue for heterotypic docking between Cx32 and Cx26 hemichannels.

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Calcium Permeability of Purified and Reconstituted Hemichannels formed by Connexin 26 and the Deafness-Causing Mutant R75W

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Gap-junctional channels are connexin oligomers (dodecamers) formed by head-to-head docking of two hemichannels (each one a connexin hexamer). Mutations of connexin 26 (Cx26) are the most frequent cause of genetic deafness. A single amino-acid mutation at position 75 (R75W) causes autosomal dominant deafness, and we have shown that this mutant is incapable of forming gap-junction channels, but forms functional hemichannels. Here, we studied the permeability of purified Cx26 and R75W hemichannels to Ca^{2+} and large hydrophilic solutes to further our understanding of the deafness associated with R75W expression. Cx26 and R75W were purified based on the affinity for Co^{2+} of a poly-histidine tag fused at the C-terminal end of the proteins overexpressed using the insect cell/baculovirus expression system, and the Cx26 and R75W affinity for the strong cation exchanger Mono S. Essentially all Cx26 and R75W solubilized in dodecylmaltoside were hexamers, and were functional when reconstituted in liposomes, as demonstrated by sucrose- and fluorescent-probe permeability assays. Cx26 and R75W hemichannels were permeable to sucrose, Alexa Fluor 350 and Cascade Blue, but not to Calcein, Fluo-5N or Alexa Fluor 647. Ca^{2+} permeability was evaluated in liposomes containing Cx26 or R75W hemichannels. In the proteoliposomes with the low-affinity calcium-sensitive probe Fluo-5N trapped inside, intra- and extra-liposomal free calcium equilibrated rapidly upon increasing free-[Ca^{2+}] from $<$ 10 nM to ~400 μM. These studies directly show permeability of Cx26 and R75W hemichannels to Ca^{2+}, but failed to identify permeability differences between hemichannels formed by Cx26 and the deafness-causing mutant. This work was supported in part by NIH grants R01GM79629 and R21DC007150, American Heart Association Grant-in-Aid 0755002Y, and a grant from the Center for Membrane Protein Research of TTUHSC.

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Origin and Dynamics of Calcium Waves in the Islet of Langerhans

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Interactions between cells in the islet of Langerhans are critical for the regulation of insulin secretion. Here, we study the electrical coupling and electrical dynamics between β-cells in the islet. We focus on quantitatively measuring multi-cellular calcium oscillations and waves, describing the emergence of these dynamics with a multi-cellular mathematical model, and experimentally testing predictions given by this model.

A key prediction is that calcium waves emerge from sub-regions of the islet with elevated excitability, due to endogenous β-cell heterogeneity in cellular excitability [Benninger et al. *Biophys.J* **95:**p5048 (2008)]. To test this we utilize a 2-channel microfluidic device [Rocheleau et al. *PNAS* **101:**p12899 (2004)] which allows a precise pattern of glucose stimulation to be applied. If glucose is elevated on one side of the islet, calcium waves consistently originate from this region, as predicted. Subsequently, upon uniform glucose stimulation, the wave direction is independent of where glucose was initially elevated: consistent with an intrinsic β-cell heterogeneity that determines calcium wave propagation.

We further explore the balance of β-cell excitability and coupling by describing the calcium activity upon a glucose gradient. In normal islets, a sharp transition in calcium activity occurs midway across the islet. In the absence of gap junction coupling this transition point is shifted towards the quiescent side of the islet. Interestingly following elevated glucose, the initial calcium elevation propagates further across the islet than subsequent calcium waves. We can explain this given a measured glucose dependence of gap-junction coupling.

This gives us further insight into the role of electrical coupling in determining the overall spatiotemporal electrical response. Furthermore the application of mathematical models to predict phenomena which can be experimentally verified to yield new functional insight is a significant advance in understanding emergent multi-cellular behavior.

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Transcriptional Suppression of Connexin 43 by Tbx18 Undermines Cell-Cell Electrical Coupling in Postnatal Cardiomyocytes

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During embryonic development, critical steps in cardiac lineage specification are guided by T-box transcription factors. Mesenchymal precursor cells expressing Tbx18 give rise to the heart's pacemaker, the sinoatrial node (SAN). We sought to identify targets of Tbx18 transcriptional regulation in the heart by forced adenoviral overexpression in postnatal cardiomyocytes. Monolayers of neonatal rat cardiomyocytes (NRCMs) transduced with GFP alone showed sarcolemmal, punctate Cx43 expression. In contrast, Tbx18-transduced NRCMs exhibited sparse Cx43 expression. Both the transcript and protein levels of Cx43 were greatly downregulated within 2 days of Tbx18-transduction. Injection of Tbx18 in the guinea pig heart *in vivo* markedly suppressed Cx43 expression similar to what was seen in Tbx18-NRCMs. The repressor activity of Tbx18 on Cx43 was highly specific: protein levels of Cx45 and Cx40, which comprise the main gap junctions in the SAN and conduction system, were unchanged by Tbx18. A reporter-based promoter assay demonstrated that Tbx18 directly represses the Cx43 promoter. Phenotypically, Tbx18-NRCMs exhibited slowed calcein dye transfer kinetics (421 ± 54 vs. control 127 ± 43 ms). Intracellular Ca^{2+}-oscillations in control NRCMs monolayers were highly synchronized. In contrast, Tbx18-overexpression led to episodes of spontaneous, asynchronous Ca^{2+}-oscillations demonstrating reduced cell-cell coupling. The decreased electrical coupling led to slow electrical propagation; conduction velocity in Tbx18-NRVMs slowed by more than 50% relative to control (4 ± 1 vs 9 ± 2 cm/s). Taken together, Tbx18 specifically and directly represses the transcript and protein levels of Cx43 in NRCMs *in vitro* and in adult ventricular myocardium *in vivo*. Cx43 suppression led to significant electrical uncoupling, but the preservation of other gap junction proteins (Cx45 and Cx40) permitted action potential propagation at slower velocity. Thus, Tbx18 overexpression recapitulates a key phenotypic hallmark of the SAN, namely the characteristic loose electrical coupling.